2,800-106 GFR:lam

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

		RECEIVED
In re Application of)	15 APR 2002
GJELSNES, Oddbjorn)	Loga, c International Division
Serial No. Not Yet Assigned)	PCT International No.: PCT/NO00/00286
Filed: Herewith)	International Filing Date: September 1, 2000
For: METHOD AND DEVICE FOR COUNTING CELLS IN URINE)	

PETITION FOR REVIVAL OF AN APPLICATION FOR PATENT ABANDONED UNINTENTIONALLY UNDER 37 CFR 1.137(b)

Attention: Office of Petitions Assistant Commissioner for Patents Box DAC Washington, D.C. 20231

Dear Sir:

The above-identified application became abandoned for failure to timely file the Request for Entry in the U.S. National Phase of International Application No. PCT/NO00/00286. The date of abandonment is the day after the expiration date of the period set for reply in the Office notice or action plus any extensions of time actually obtained.

02/27/2002 MNGUYEN 00000153 10069044

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640.00 OP

	APPL:	ICANT	HEREBY PETITIONS FOR REVIVAL OF THIS APPLICATION.
1.	Peti	tion f	ee
	X		small entity - fee \$640.00. Applicant claims small entity status.
			other than small entity - fee \$1,280.00.
2.	Repl	y and/	or fee
	Α.		ee for entering into the U.S. National Phase for 1000/00286 is
			has been previously filed on
		X	is enclosed herewith.
	В.	The	issue fee of \$
			has been paid previously on
			is enclosed herewith.
3.	Term	inal o	disclaimer with disclaimer fee
	X		Since this utility/plant application was filed on or after June 8, 1995, no terminal disclaimer is required.
			A terminal disclaimer (and disclaimer fee of \$55.00 for a small entity of \$110.00 for other than a small entity) disclaiming the required period of time is enclosed herewith (see PTO/SB/63).
4.	STATEM from t a grar (NOTE.	the due	The entire delay in filing the required reply e date for the required reply until the filing of petition under 37 CFR 1.137(b) was unintentional.

additional information if there is a question as to whether either the abandonment or the delay in filing a petition under 37 CFR 1.137(b) was unintentional

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

Respectfully submitted,

Ву

G. Franklin Rothwell

Attorney for Applicants Registration No. 18,125

ROTHWELL, FIGG, ERNST & MANBECK, p.c.

Suite 701-E, 555 13th Street, N.W.

Washington, D.C. 20004

Telephone: (202)783-6040

JC20 Rec'd PCT/PTO 2 1 FEB 2002 U.S. Department of Commerce Patent and Trademark Office Attorney's Docket No. TRANSMITTAL LETTER TO THE UNITED STATES 2800-106 DESIGNATED/ELECTED OFFICE (DO/EO/US) U.S. Application No. (1 known, sec 37 CFR 1 5) **CONCERNING A FILING UNDER 35 U.S.C. 371** N101069044 INTERNATIONAL APPLICATION NO. INTERNATIONAL FILING DATE PRIORITY DATE CLAIMED PCT/NO00/00286 September 1, 1999 September 1, 2000 TITLE OF INVENTION METHOD AND DEVICE FOR COUNTING CELLS IN URINE APPLICANT(S) FOR DO/EO/US GJELSNES, Oddbjorn et al. Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information: [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371 [] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. The US has been elected by the expiration of 19 months from the priority date (Article 31). [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2)) a. [X] is attached hereto (required only if not communicated by the International Bureau). b. [] has been communicated by the International Bureau. c. [is not required, as the application was filed in the United States Receiving Office (RO/US) An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). a. [] is attached hereto. b. [] has been previously submitted under 35 U.S.C. 154(d)(4). [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) a. [] are attached hereto (required only if not communicated by the International Bureau). b. [] have been communicated by the International Bureau. c. [] have not been made; however, the time limit for making such amendments has NOT expired. d. [X] have not been made and will not be made. An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). 10. [] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). ITEMS 11. TO 20. below concern other document(s) or information included:] An Information Disclosure Statement under 37 CFR 1.97 and 1.98. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. [X] A FIRST preliminary amendment. 14. [] A SECOND or SUBSEQUENT preliminary amendment. A substitute specification. 16. [A change of power of attorney and/or address letter. A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825] A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. [] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. [X] Other items or information: Petition to Revive with fee check

U.S. APPLICATION NO. ((gknown,	6'90'44	INTERNATIONAL APPLICA PCT/NO00/00286	TION NO	ATTORNEY DOCKET N 2800-106	40
21. [X] The following fee Basic National Fee (37 C Neither international preli- nor international search for International Search Report International preliminary but International Search F	es are submitted. FR 1.492(a)(1)-(5): Iminary examination fee (37 CFR 1 445(a)(2)) ort Not Prepared by EPC examination fee (37 CI Report has been prepare examination fee (37 CFR 1.445(a)(2)) examination fee (37 CI provisions of PCT Artiexamination fee (37 CI Removed for the examination fee (37 CI provisions of PCT Artiexamination fee (37 CI Removed for the examination fee (37 CI Removed for the examin) paid to USPTO and O or JPO R 1.482) not paid to USPTO d by the EPO or JPO R 1.482) not paid to USPTO) paid to USPTO R 1 482) paid to USPTO cle 33(1)-(4) R 1.482) paid to USPTO	\$ 890 00	CALCULATIONS	PTO USE ONLY
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Surcharge of \$130.00 for fur months from the earliest clai			20 [] 30	\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	7 -20 ≈	0	X \$18.00	\$0	
Independent Claims	2 -3=	0	X \$84.00	\$0	
Multiple dependent claim(s)) (if applicable)	,	+ \$280.00	\$0	
		TOTAL OF ABOVE CA	LCULATIONS =	\$ 1,040.00	
Applicant claims small above are reduced by by		7 CFR 1.27. The fees indi	cated	\$ 520.00	
<u> </u>		·	SUBTOTAL =	\$ 520.00	
Processing fee of \$130.00 for months from the earliest claim			[]20[]30 +	\$	
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Customer No. 6449			_	www.tl	
G. Franklin Rothwell, Esq. Rothwell, Figg, Ernst & Manbe	ock		G. Franklin Roth	iweii	
555 13th St., N W Washington, D.C 20004 Phone: 202/783-6040			18,125 Registration Number	er	

APPLICATION DATA SHEET

Inventor Information

Inventor One Given Name::

Oddbjorn

Family Name::

GJELSNES

Name Suffix::

Postal Address Line One::

Gladvoll terrasse 2

Postal Address Line Two::

City::

Oslo

State or Province::

Country::

Norway

Postal or Zip Code

N-1168

Citizenship Country:: Norway

Inventor Two Given Name::

Oystein

Family Name::

RONNING

Name Suffix::

Postal Address Line One::

Somveien 7B

Postal Address Line Two::

City::

Oslo

State or Province::

Country::

Norway

Postal or Zip Code

N-0493

Citizenship Country::

Norway

Correspondence Information

Correspondence Customer Number:: 6449

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Application Information

Title Line One::

METHOD AND DEVICE FOR COUNTING CELLS IN

Title Line Two::

URINE

Total Drawing Sheets::

ONE (1)

Formal Drawings?::

YES UTILITY

Application Type::
Docket Number::

2800-106

Secrecy Order in Parent Appl?::

NO

Representative Information

Representative Customer Number:: 6449

Prior Foreign Applications

Foreign Application One::

19994228

Filing Date::

September 1, 1999

Country::

Norway

Priority Claimed::

Yes

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	PCT/NO00/00286
Filing Date	September 1, 2000
First Named Inventor	GJELSNES, Oddbjorn
Group Art Unit	Unassigned
Examiner Name	Unassigned
Attorney Docket Number	2800-106

Title of the Invention:

METHOD AND DEVICE FOR COUNTING CELLS IN URINE

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the above-referenced application as follows:

Amend claims 4, 5 and 6 as shown on the following pages.

Marked-up copies of the original text of the amended claims are attached to this amendment. Material inserted is indicated by underlining and material deleted is indicated by brackets.

PCT/NO00/00286 Page 2

Clean Copy of Amended Claims

- 4. (Amended) A method according to claim 1, characterized in that the dye used is a flurochrome that specifically attaches to the nucleic acids of the cells, and that it is monomer cyanine fluorochrome, preferably TOPRO-3.
- 5. (Amended) A method according to claim 1, characterized in that the mixture is analyzed in a device that measures light scatter and fluorescence from the individual cells, such as a flow cytometer.
- 6. (Amended) A method according to claim 1, characterized in that the analyses are preformed at a wave length >500, preferably at 635 nm.

REMARKS

The amendments to the claims are made to correct improper claim dependencies and to put the claims in the format preferred by the U.S. Patent Office. No new matter is introduced by means of these amendments.

	RESPECTFULLY SUBMITTED	,	
NAME AND REG NUMBER	G. Franklin Rothwell, Reg. No	. 18,12	25
SIGNATURE	G. F. lowers	DATE	2.21.0z

Address	Rothwell, Figg,	Rothwell, Figg, Ernst & Manbeck				
	Suite 701-East,	555 13th Street,	N.W.			
City	Washington	State	D.C.	Zip Code	20004	
Country	U.S.A.	Telephone	202-783-6040	Fax	202-783-6031	

Attachments: Marked-Up Copies of Amendments to the Claims

PCT/NO00/00286 Page 3

Amended Claims: Version with markings to show changes made

- 4. (Amended) A method according to claim[s] 1 [-2], characterized in that the dye used is a flurochrome that specifically attaches to the nucleic acids of the cells, and that it is monomer cyanine fluorochrome, preferably TOPRO-3.
- 5. (Amended) A method according to claim[s] 1 [-4], characterized in that the mixture is analyzed in a device that measures light scatter and fluorescence from the individual cells, such as a flow cytometer.
- 6. (Amended) A method according to claim[s] 1 [-5], characterized in that the analyses are preformed at a wave length >500, preferably at 635 nm.

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Method and device for counting cells in urine

The present invention regards a method and a device for counting bacteria and other micro-organisms in urine from a patient. The method and the device are very quick and accurate in terms of diagnosing cystitis. The technical area of the invention is medical diagnostics. The techniques used are covered by the areas of biochemistry/microbiology, optics, fluid mechanics, electronics and computer science. The novel aspects of the invention fall mainly within the subjects of biochemistry and microbiology.

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The invention involves detecting bacteria by means of light scatter and fluorescence with an improved signal-to-noise ratio when compared with prior art.

Persons suffering from cystitis have cells in their urine that should not normally be there. These cells in the urine may be bacteria and fungus, as well as the patient's own cells (somatic cells), such as leukocytes or epithelial cells.

The invention seeks, through the method and device thereof, to solve the following problem: In the case of cystitis, it takes a long time (one or more days) to count the number of bacteria in urine. This because the bacteria must be cultivated on agar discs until they form macroscopic colonies than can be seen with the naked eye. The long wait required before a diagnosis can be made is unfortunate, as the patient is often given antibiotics before a certain diagnosis has been made.

Attempts have been made to solve this problem by counting the bacteria and other cells (lymphocytes and epithelial cells) directly in the urine by using specially designed cell counting devices (flow cytometers). In order to be able to do this, the cells must be made fluorescent by adding special fluorochromes that attach to the cells. In the flow cytometer, the cells are illuminated by a beam of light as they pass the measurement point in a liquid stream (thus flow cytometry). The instrument registers the light scatter and fluorescence from each individual cell. The intensity of the scattered light is a function of among other things the size of the cell, and the intensity of the fluorescent light is a function of among other things the amount of substance made fluorescent (e.g.

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nucleic acids). The concentration of cells (number of cells per ml urine) is simply determined by counting the number of fluorescent particles in the sample. This may be grouped into different types of cells based on the size of the cells (light scatter) and the content of nucleic acids (fluorescence). There are also other quick methods of measuring bacteria in urine, however these are indirect and measure the presence of cellular metabolites (dipsticks).

The main problem associated with prior art that makes use of plate counting is the time it takes. The problem with today's flow cytometers is that they are not good enough at routinely measuring bacteria in urine, which are small in comparison with somatic cells (lymphocytes, epithelial cells).

US 5 693 484 regards a method of counting and classifying cells in urine. A fluorescent dye is added to the urine sample, which dye attaches to the nucleic acids of the cells. The cells are then illuminated with light at the blue and violet wavelengths, and analysed in a flow cytometer.

The method according to US 5 639 484 functions satisfactorily with somatic cells, but does not work well with bacteria. This is, among other things, due to the following facts:

- Using violet/blue excitation light results in auto-fluorescence, which causes
 the signal-to-noise ratio to be reduced at low fluorescence intensities (as in
 the case of bacteria).
- It is more difficult for live bacteria to absorb dye than it is for somatic cells, for several reasons.
- Firstly, the cell walls of the bacteria act as a barrier against the surroundings.
- Secondly, the bacteria may have intracellular pumps that bring the dye out again.
- Thirdly, the bacteria are considerably smaller than somatic cells, thus containing less of the cellular components that are to be stained.
- As a result of this, the fluorescence intensity per cell is low.

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The present invention provides a method and a device that are reliable and quicker than the known techniques. The method consists of the following steps:

- 1. The urine sample from the patient is undiluted and is mixed with a fixative liquid so as to kill all the cells. The fixative liquids that may be used must be such that they render the cellular membrane permeable for absorption of the dyes (fluorochromes) mentioned below. Fixatives that may be used include ethanol, isopropanol and acetone, acetone being particularly preferred.
- 2. The mixture from point 1 has a buffer solution added to it, which is formulated so as to promote attachment of fluorochrome to the nucleic acids of the cells (DNA/RNA) (see point 3). At the same time, the buffer solution must prevent attachment to other cellular components. The buffer that has been found to be the most optimal is the so-called TBE-buffer (90 mM Tris, 90 mM Borate, 2,5 mM EDTA, pH 8).
- 3. A fluorochrome is added to the mixture from point 2, which fluorochrome specifically attaches to the nucleic acids of the cells. The present method may for instance involve the use of a monomer cyanine fluorochrome.
- 4. The mixture from point 3 is analysed in a device that measures light scatter and fluorescence from individual cells (e.g. a flow cytometer). The excitation light has a wavelength (635 nm) such that auto-fluorescence from the cells is insignificant.
- 5. The results are presented on a display that shows the fluorescent particles (cells) appearing separately (different colour) from particles without fluorescence, while displaying the absolute count. Cells in the lower size range (0.5 2μm) are assumed to be bacteria.
- 6. Steps 1 5 can be performed by a novel device according to the invention, such as appears in the accompanying schematic figure.

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More specifically, the invention regards a method for counting cells in a urine sample, characterised in that a fixative is added to and mixed with the urine sample; a buffer solution is added to the mixture; followed by a dye; the mixture is then analysed in a device that measures light scatter and fluorescence from individual cells; and the results are shown directly on a display.

The invention further regards a device for measuring cells in a liquid stream by means of flow cytometry, in particular bacteria in a urine sample, characterised in that it comprises pickup tubes for the urine sample, which tubes lead to one or more mixing chambers to which are also connected separate receptacles for the fixative and the staining solution that are added to the mixing chamber via adjustable multi-channel pumps; the mixing chamber is further connected to an optical flow cytometric cell that receives carrier liquid from a receptacle.

According to the method of the invention, fluorescence is achieved by staining the bacteria. The cellular membrane is broken down when the cell is fixed by a fixative liquid such as ethanol, isopropanol or preferably acetone. The fixation also inactivates any eflux-pumps that may otherwise pump the dye back out of the cells. In this manner, the fluorochrome gains easy access to the intracellular components of the cells.

A further advantage is the fact that the method prevents auto-fluorescence by use of a dye that attaches specifically to nucleic acids and which is excited at light >500 nm (specifically 636 nm). The gain in fluorescence increases >10x upon attachment to the nucleic acids.

The method promotes specific attachment and reduces non-specific attachment by utilising special buffers, and the use of Tris-borate-EDTA, pH 8 has proven to be especially advantageous.

The device according to the invention, which may be used to implement the method, is explained schematically in greater detail in Figure 1.

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The device consists of a connection for inlet of urine from a sampling bottle 1. The urine sample is sucked in by pump 2, and the sample is passed on to a mixing chamber or a reagent loop 5. A fixative such as ethanol or acetone is introduced into the mixing chamber 5 by pump 4. The staining solution is kept in receptacle 6 and is led to mixing chamber/reagent loop 8 by pump 7. A common motor 18 can drive pumps 2, 4, 7.

After the mixing has been completed in chamber 8, biological and chemical waste is separated out in a separate receptacle 10. The mixture of the urine sample, the fixative and the staining solution is sent on to the flow cell 11, in which the optical detection takes place. Light scatter is detected using MICROCYTE (Norwegian, European, US patent, pending Japan). For detection in the flow cell, use is made of a carrier liquid from receptacle 12. The amount and velocity of the carrier liquid 12 is adjusted by means of e.g. a throttle valve 9. Following detection of the sample in the flow cell 11, it is sent to waste container 14 by pump 13, which is connected to motor 17. This waste consists mainly of water with a very low content of biological material and chemicals.

The measurement of the urine sample in the flow cell is transferred to a data and control unit, where the results are shown on a display. The results are presented on a display where the fluorescent cells appear separately with a different colour from that of non-fluorescent particles. In addition, the total cell count is shown on the display. Cells in the lower size range from 0.5 to 2 µm are presented as bacteria.

The method and device according to the invention have a number of advantages over prior art, including the fact that they allow quicker and more reliable counting of bacteria in urine.

Using today's conventional plate technique, in which cultivated colonies of bacteria must be determined and counted using the naked eye, the analysis may take from one to several days, and may often require the sample to be sent away for analysis. By using the method and the device of the invention, the results of the analysis are available on site in a matter seconds.

A great advantage of the device is the fact that it is automated. There is no manual handling of chemicals, which removes the risk of the operator being exposed to any chemicals that may be injurious to his or her health.

The device also ensures a reduced possibility of human errors and failures during the handling and treatment of the sample.

By using the method and the device of the invention, the cost per sample will be lower than that which is the case for the conventional methods of analysis that are in use today.

Claims

1.

A method for counting cells in a urine sample,

characterised in that

- a fixative is added to and mixed with the urine sample;
- a buffer solution is added to the mixture, followed by a dye;
- the mixture is analysed in a device that measures light scatter and fluorescence from individual cells; and
- the results are shown directly on a display.

2.

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A method according to Claim 1,

c h a r a c t e r i s e d i n that the fixative is of the type that renders the cellular membrane permeable, and may be acetone, ethanol or isopropanol, preferably acetone.

3.

A method according to Claim 1,

c h a r a c t e r i s e d i n that the buffer solution promotes attachment to the nucleic acids of the cells, and that it is preferably a TBE-buffer consisting of 90 mM Tris, 90 mM Borat, 2,5 mM EDTA, pH 8.

4.

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A method according to Claims 1-2,

c h a r a c t e r i s e d i n that the dye used is a fluorochrome that specifically attaches to the nucleic acids of the cells, and that it is a monomer cyanine fluorochrome, preferably TOPRO-3.

5.

A method according to Claims 1 - 4,

c h a r a c t e r i s e d i n that the mixture is analysed in a device that measures light scatter and fluorescence from the individual cells, such as a flow cytometer.

6.

A method according to Claims 1-5,

c h a r a c t e r i s e d i n that the analyses are performed at a wave length >500, preferably at 635 nm.

7.

A device for measuring cells in a liquid stream by means of flow cytometry, particularly bacteria in a urine sample,

c h a r a c t e r i s e d i n that it comprises pickup tubes for a urine sample (1), which tubes lead to one or more mixing chambers (5, 8) to which are also connected separate receptacles for a fixative (3) and a staining solution (6) that are added to the mixing chamber (5, 8) via adjustable multi-channel pumps (2, 4, 7, 9), the mixing chamber further being connected to an optical flow cytometric cell (11) to which is added a carrier liquid from receptacle (12).

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



LIBBAR DIKINDERU BIDIK DENGKAN KANDAN BIRA DIKA DIKA DIKA DIKA DIKA DIKA DIKAN DIKAN DIKAN DIKAN DIKAN BIRA KA

(43) International Publication Date 8 March 2001 (08.03.2001)

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- (71) Applicant (for all designated States except US): OPTOFLOW AS [NO/NO]; P.O. Box 70 Bogerud, N-0621 Oslo (NO).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): GJELSNES, Oddbjørn [NO/NO]; Gladvoll terrasse 2, N-1168 Oslo (NO) RØNNING, Øystein [NO/NO]; Sømveien 7B. N-0493 Oslo (NO).

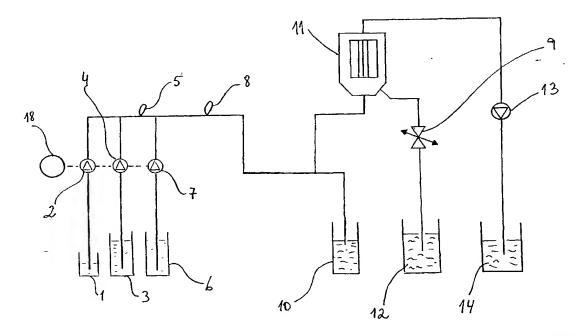
- (74) Agent: PROTECTOR INTELLECTUAL PROPERTY CONSULTANTS AS; P.O. Box 5074 Majorstua, N-0301 Oslo (NO).
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Published:

With international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette

(54) Title: METHOD AND DEVICE FOR COUNTING CELLS IN URINE

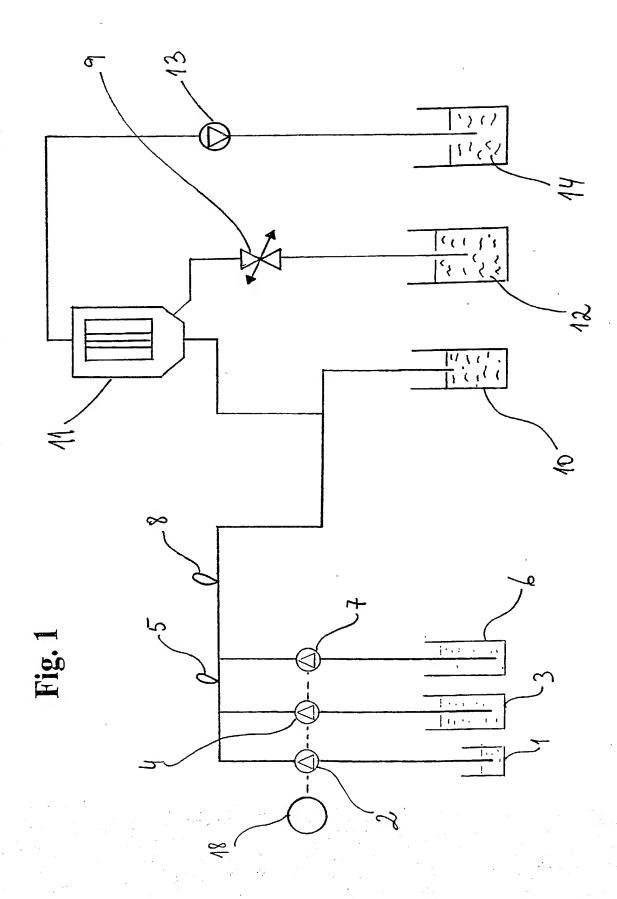


(57) Abstract: The invention regards a method and a device for measuring the number of cells in urine. A fixative, a buffer and a dye are added to the urine sample, which is then analysed in a device for measuring fluorescence.



01/16595 A1

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2800-106

Attorney Docket No.

DECLARATION AN	ND POWER	R OF	First Na		GJELSNES,	Oddbjorn	İ
ATTORNEY FOR UT	ILITY OR D			COMPLETE IF KNOWN			
PATENT APP (37 CFR			Applicat	tion Number	PCT/NO00/0	00286	
Declaration X	Declaration	n	Filing D	ate	September 1	, 2000	
Submitted with Initial	Submitted after Initial		Group A	Art Unit	N/A		
Filing	Filing		Examin	er Name	N/A		
As a below named inventor, I he	ereby declare	that:					
My residence, mailing address,	and citizensh	nip are as s	tated below	next to name.			
I believe I am the original, first a (if plural names are listed below invention entitled: METHOD AN on September 1, 2000 as PCT 2002.	v) of the subje ID DEVICE F International	ect matter v OR COUN Application	vhich is clair TING CELL: Number PC	ned and for whose IN URINE the CT/NO00/00286	icn a patent is e specification 6 and was am	of which is vended on Fe	vas filed bruary 21,
I hereby state that I have review claims, as amended by any am	ved and unde endment spe	erstand the cifically ref	contents of erred to abo	the above iden ve.	tified specifica	ation, includir	ig the
I acknowledge the duty to discler for continuation-in-part applicat application and the national or I	ions materia	l informatio	n which bec	ame avallable	pelween the ii	iling date of the	ncluding he prior
I hereby claim foreign priority be inventor's certificate, or 365(a) the United States of America, lifer patent or inventor's certification which priority is claimed.	of any PCT it	nternationa nd have als	l application so identified	which designated below, by check	ted at least or king the box,	ne country of any foreign a	ner man
Prior Foreign Application Numbers	Country		Filing Date D/YYYY)	Priority Not Claimed	Certified C YES	Copy Attache NO	d?
19994228	Norway	09/01/199	99				
I hereby claim the benefit under	er 35 U.S.C. 1	19(e) of ar	ny United Sta	ates provisiona	l application(s	i) listed below	v.
Application Nur					(MM/DD/YYY		

I or we hereby appoint the registered practitioner(s) associated with Customer No. **6449** to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. Direct all correspondence to

Customer Number 6449.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR:	[] A petition l	nas been filed for this un	signed inventor	
Given Name (first and middle [if any]) Oddbjorn.		Family Name or Surname <u>GJ</u>	ELSNES	
Inventor's Signature Oddy	Giden	Date 26 -	02-2007	
Residence: City Oslo	State NOX	Country Norway	Citizenship Norway	
Mailing Address Gladvoll terrasse 2				
Mailing Address				
City Oslo	State	Zip N-1168	Country Norway	
NAME OF SECOND INVENTOR:	[] A notition by	has been filed for this unsigned inventor		
NAME OF SECOND INVENTOR.	[] A petition ha	as been filed for this uns	aigned inventor	
Given Name (first and middle [if any]) Oystein	7) A petition is	Family Name	onning	
Given Name	Roules	Family Name or Surname R		
Given Name (first and middle [if any]) Oystein Inventor's Signature	D	Family Name or Surname R	onning	
Given Name (first and middle [if any]) Oystein Inventor's Signature	Roueles	Family Name or Surname Ronger 27 - 07	onning	
Given Name (first and middle [if any]) Inventor's Signature Residence: City Oslo Oystein Oystein	Roueles	Family Name or Surname Ronger 27 - 07	onning	